Padilla Bay
National Estuarine Research Reserve

Technical Report No. 36

Science Presentation Abstracts

From the workshop on

The Science and Management of *Zostera japonica* in Washington: A meeting for state agencies

Compiled by

Douglas A. Bulthuis

June 2013

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WASHINGTON STATE DEPARTMENT OF ECOLOGY

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION NOAA U.S. DEPARTMENT OF COMMERCE

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June 18 – 19, 2013
Lacey, Washington

Compiled by

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Introduction

In response to concern about the non-native eelgrass, *Zostera japonica*, in Washington State, a meeting on the science and management of *Z. japonica* was organized for June 18 – 19, 2013 in Lacey, Washington. "The Science and Management of *Zostera japonica* in Washington: A Meeting for State Agencies" was co-sponsored by the Washington State Departments of Ecology, Natural Resources, and Fish and Wildlife, and by the Washington State Invasive Species Council. The science presentations and science panel were organized by D. Bulthuis, Padilla Bay National Estuarine Research Reserve, Department of Ecology and F. Short, Nearshore Habitat Program, Department of Natural Resources.

The meeting included presentations by scientists on the biology, ecology, and impacts of *Z. japonica* in the Pacific Northwest and a report by a science panel. In addition to the presentations at the meeting, the science presenters were asked to submit an extended abstract with references to the scientific literature. The present report includes the extended abstracts and references prepared by the science presenters. A meeting description and agenda are included in an appendix to the present report to provide the reader with the context for these abstracts and references. A separate report on the issues and recommendations of the scientific panel has been prepared by F. Short, Department of Natural Resources.

The 2010 *Zostera japonica* workshop: An overview

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On September 23 and 24, 2010, a workshop was hosted at the Friday Harbor Laboratories, University of Washington, San Juan Island, WA, to address the distribution and potential effects of a non-native seagrass in Washington State. The workshop was funded by the Washington State Department of Natural Resources and Washington Sea Grant and included participants from academia and state and federal agencies from the US and Canada. Two goals of the workshop were 1) to convene scientists and managers to discuss and synthesize the best available knowledge on *Z. japonica*, and 2) to identify research priorities that will enhance the current knowledge of *Z. japonica* and improve the management of seagrass in the region. Specific topics were assigned to selected participants and data were synthesized and presented during the workshop. Each presentation was followed by a discussion to clarify findings, incorporate overlooked data and identify data gaps for future research priorities. Presentation topics included: taxonomic history of *Z. japonica* in the Pacific Northwest, current regulatory status of *Z. japonica* in WA state, the effects *Z. japonica* on ecosystem structure and function, community and species level interactions involving *Z. japonica*, monitoring *Z. japonica* distribution and expansion, climate change effects on *Z. japonica* and the genetic variation within and among *Z. japonica* populations. As a result of the presentations, participants identified the following research priorities: continue to synthesize *Z. japonica* research literature, encourage citizen monitoring, investigate the effects *Z. japonica* has on community dynamics and ecosystem functions over a range of temporal and spatial scales, assess the economics of *Z. japonica*, and conduct additional genetic analyses to confirm its non-native status and its response to global climate change. Workshop findings were summarized in a document found at this link - http://www.dnr.wa.gov/Publications/aqr_zostera_study.pdf.

Mach, Megan E., Sandy Wyllie-Echeverria, and Jennifer Rhode Ward. 2010. *Distribution and potential effects of a non-native seagrass in Washington State:* Zostera japonica *workshop*. Report for the Washington State Department of Natural Resources and Washington Sea Grant on the *Zostera japonica* Workshop, September 23-24, 2010, Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington.

Zostera japonica: What is it and Where is it?

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Seagrasses are flowering plants from the monocot order Alismatales that returned to the marine environment between 17 and 75 million years ago (1). Seagrasses form an ecological group, not a taxonomic group (2), and as a result they encompass a variety of species characterized by adaptations to the marine environment (e.g. salt tolerance, underwater pollination, clonal growth, specialized leaves, etc.). Seagrass communities provide important ecosystem services (e.g. 3- dimensional habitat, primary production, nutrient removal, Ocean Acidification amelioration) which can contribute $3500 to $19000 ha-1 y-1 (3, 4). Seagrass populations worldwide are experiencing declines at a rate of about 110 km2 y-1 and ~30% of seagrass areal extent has disappeared (3). The Pacific Northwest is one of a few places experiencing increased seagrass areal distribution and one of only two places known to have non-native seagrasses (5, 6).
Six seagrass species occur in Washington State (7). The dominant species based on areal extent are the native *Zostera marina* L. and non-native *Z. japonica* Aschers. & Graebn. Early descriptions of *Z. japonica* in North America were confounded by taxonomic uncertainty, morphological plasticity and contradictory descriptions of leaf-tip morphology, a key diagnostic feature. Early synonymous identifications have included *Z. nana*, *Z. noltii* and *Z. americana*. Researchers (2, 8) have concluded that the genus *Zostera* should be divided into subgenera and that *Z. japonica* be recognized under the subgenus *Zosterella*. Currently, *Z. japonica* is the recognized nomenclature, although recent genetic analyses indicate more work is needed (9).

Z. japonica is believed to have been introduced to North America with oysters during the early 20th century. Harrison (10) cites personal communication with R. Scagel indicating that oysters may have been packed with eelgrass (species unknown), similar to the introduction of *Sargassum muticum*. The first large-scale introductions of Pacific oysters (*Crassostrea gigas*) from Miyagi Prefecture, Japan to Samish Bay in Puget Sound began in 1919 (11). In the early 1950's steps were taken to prevent accidental introduction of other organisms (12); consequently, it is likely that *Z. japonica* was introduced before the 1950's (13). The oyster- *Z. japonica* vector hypothesis is supported by genetic studies that indicate *Z. japonica* from British Columbia was strongly related to samples from Miyagi-Ishinomaki, Japan (14).

Within its native range, *Z. japonica* has an extremely broad latitudinal distribution, encompassing subtropical and temperate climates from southern Vietnam (~10º N latitude) to Kamchatka, Russia, (~50 º N latitude) (15, 16). Currently, *Z. japonica* has been reported from the Eel River, Humboldt County, California (40.6° N) at the southern end of its distribution almost to Campbell River, British Columbia (49.9° N; 17, 18) to the North. The earliest known collections of *Z. japonica* were from September 1957 at "south-east end of Long Island" in Pacific County, WA (19). Additional samples were collected from Padilla Bay, Boundary Bay and Yaquina Bay during the 1970's (20, 21). In 2002, *Z. japonica* was reported from Indian Island in Humboldt Bay, CA (22). *Z. japonica* has been reported from most estuaries in Oregon and Washington (23, 24). Genetic analyses indicate that *Z. japonica* can be separated into populations with warm water and cold water affinities (14).

In its native range, *Z. japonica* has been reported to grow as deep as 3-7 m (datum not specified), although it typically grows at depths < 1 m (25, 26). Within colonized PNW estuaries, *Z. japonica* exhibits a distribution pattern that tends to minimize interactions with the native *Z. marina*. *Z. japonica* is found primarily in mid- to upper- intertidal zones, and has not been observed growing sub-tidally. In California, *Z. japonica* has been reported to occur between +0.9 and +1.2 m Mean Lower Low Water (MLLW) (22). In Oregon, *Z. japonica* typically occurs between +1 to +3 m MLLW (27). In Willapa Bay, Washington, *Z. japonica* was documented between +0.1 to +1.5 m MLLW, while *Z. marina* was only found < +0.6 m MLLW (28). In contrast, *Z. japonica* in Puget Sound has been found as deep as 0 m MLLW (29). Reports from British Columbia indicate it generally occurs between +1 to +3 m MLLW (30, 31).

In places where *Z. marina* and *Z. japonica* co-occur there are three distinct vertical zonation patterns (32). In the disjunct zonation, the *Z. japonica* bed is separated from the *Z. marina* bed by unvegetated sediments. These areas are characterized by a steep intertidal slope and a narrow fringing *Z. japonica* bed. The overlapping zonation pattern is characterized by mixed beds or discrete patches of both species at the same intertidal elevation. Overlapping zonation has been observed at sites with gently sloping topography. The mosaic zonation pattern is characterized by micro- topographic relief creating small pools with *Z. marina* interspersed with *Z. japonica* on well-drained hummocks. Mosaic sites, which often co-occur with the overlapping zonation pattern, are characterized by broad, expansive intertidal flats with very little slope (32, 33) and are generally localized in larger estuarine systems such as Boundary Bay, Padilla Bay, and Willapa Bay.

Physiological studies indicate *Z. japonica* is both euryhaline and eurythermal, with a lethal chronic temperature threshold between 32-35 ºC (34, 35). Assuming that transport vectors remain active, it is likely that, *Z. japonica* will continue to spread to the south until it reaches systems that regularly exceed its environmental tolerances (36, 37). Additionally, rising water temperatures expected to occur with global climate change may facilitate the northern expansion of *Z. japonica*. Consequently, it is likely that the distributional range of *Z. japonica* along the Pacific Coast of North America will continue to expand.

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Role and Growth of *Zostera japonica* in its Native Range

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There are 72 species of seagrass distributed throughout the world, which have been grouped into six bioregions based on species distribution and temperate and tropical boundaries (Short et al. 2011). *Zostera japonica* in its native range is found only in Asian coastline of the Pacific Ocean, where it occurs in both the North Pacific Temperate Bioregion and the Tropical Indo-Pacific Bioregion (Short et al. 2007). *Zostera japonica* is one of few seagrass species that thrive in both temperate and tropical environments, extending in Asia from the Russian Federation in the north to southern Vietnam in the south. In North America it is one of six seagrass species found on the North Pacific coast, where it is believed to be an introduced species, and it is distributed from northern British Columbia to north central California. As part of SeagrassNet, the global seagrass monitoring program, five sites are monitoring *Z. japonica*, three in Asia and two in North America. At the SeagrassNet site in South Korea, beds of *Z. japonica* and *Zostera marina* are found growing adjacent to one another and intermixed, similar to what is seen in Puget Sound. In both Asia and North America *Z. japonica* occupies the mid-intertidal. As in Washington State, in Asia seagrass meadows and bivalve aquaculture utilize the same areas of the intertidal and shallow subtidal coast. In South Korea conflict over the use of the intertidal has been minimized by employing off-bottom oyster aquaculture and the rotational harvesting of clam species including the Manila clam (*Ruditapes philippinarum*). Park et al. (2011) show the growth and biomass of *Z. japonica* before and after intense mechanical harvest of Manila clams in a South Korean estuary. They conclude that rotational harvest of Manila clams meets the needs of the aquaculture industry while allowing normal vegetative regrowth of *Z. japonica*. *Zostera japonica*, as all other seagrasses, is an underwater flowering plant, which reproduces sexually, through seed production, as well as vegetatively through rhizome expansion. At a SeagrassNet site in southern China, *Z. japonica* grows extensively in bivalve aquaculture areas, and regrows vegetatively and from seed after clam harvesting. In Asia, *Z. japonica* and aquaculture coexist and there has been no need for *Z. japonica* eradication in estuarine waters.

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Ecosystem functions of the non-native eelgrass, *Zostera japonica*, in the Pacific Northwest

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Eelgrasses and other seagrasses provide several ecosystem functions in coastal and estuarine environments throughout the world and in the Pacific Northwest (PNW)[2, 11, 21, 23, 24, 35, 37, 47]. The combined value of these functions and services has been valued in economic terms at more than $45,000 per acre per year[9]. Much of the research on these ecosystems functions has been conducted on the eelgrass, *Zostera marina*, and two other seagrasses[12]. In this talk I have selected a few of the ecosystem functions and will describe these functions in *Z. marina* and other seagrasses, present any studies indicating these functions in *Z. japonica*, and discuss the possible role of *Z. japonica* in providing these functions in the PNW.

A high rate of productivity is one of the ecosystem functions often listed and valued for seagrasses[35, 37]. Rates of productivity vary widely but can be similar to intensive agriculture, mangroves, marsh plants and some forests per unit area[12]. Annual mean above ground growth rates for *Z. japonica* in the PNW and in Asia and are in the range of 0.9 to 1.7 g dry weight m^{-2} [25, 27, 31, 32, 36, 42, 48]. These rates are in the range of rates for *Z. marina*, although in the lower part of the range, and similar to estimates of annual production for macroalgae and coral reefs[12]. Thus, one ecosystem function of *Z. japonica* in the PNW is moderate rates of productivity. Many of the other ecosystem functions will be proportional to the productivity of *Z. japonica*.

Eelgrass and seagrass productivity supports higher trophic levels primarily through the detritus pathway[23, 34, 50]. Leaves and plant parts break off, are enriched by bacteria and fungi and become food/energy for mico- and meso- organisms which, in turn support higher trophic levels such as juvenile fish and crabs[24, 45]. *Z. japonica* in the PNW also contributes to the detrital food chain with the leaf productivity[27, 48]. *Z. japonica* decomposes at a faster rate than *Z. marina* and thus may enter the detrital food chain at different rates and timing than the native eelgrass[22]. In addition to the detrital pathway, *Z. japonica* is consumed directly by native fauna in the PNW such as the isopod *Idotea* and the waterfowl Black Brant[1, 49]. In a study in Padilla Bay herbivory of both species of eelgrass by isopods, caprellids, and other grazers accounted for a significant proportion of the eelgrass productivity in the bay[49]. Thus, *Z. japonica* is a source of food for estuarine and marine species in the PNW, both via the detrital pathway and via direct herbivory.

Some of the organic production of seagrasses is exported to other systems such as adjacent salt marshes, sandy beaches, or to deep waters off shore from seagrass beds or to the more distant deep sea[26, 34, 35, 46]. The presence and use of eelgrass detritus in deep channels has been documented in Washington in the San Juan Channel[5]. *Z. japonica* would be expected to contribute to this export of organic matter in proportion to its biomass and productivity.

Recently the role of seagrasses in carbon sequestration has been reported[13, 19]. Some seagrasses, such as *Posidonia* species develop large mats of organic matter that become buried in the sediments[13]. Thus soil organic matter is the major mechanism of sequestration of carbon by seagrasses[19]. However, *Z. japonica* is much more likely to be consumed directly or indirectly or exported than for organic material to accumulate in the sediments. Thus, *Z. japonica* is unlikely to be a major contributor to this ecosystem function in the PNW.

Another group of ecosystem functions of seagrasses that are valued revolves around the increased structure that eelgrasses and seagrass leaves provide to the system in comparison to intertidal flats without macro-vegetation[30, 38]. These ecosystem functions include substrate for epiphytes, attenuation of waves and currents, settling and trapping of suspended material, retention of water on the flats during ebbing tide, and habitat for marine and estuarine fauna.

Leaves provide substrate for epiphytes[3, 37]. Numerous grazers feed on these epiphytes[43, 49]. Epiphyte growth and grazer utilization have been reported for *Z. japonica* in Asia and in the PNW[15, 30, 49]. These contribute to the overall secondary productivity of the ecosystem as well as provide suitable food for specific grazers. In the PNW, epiphyte growth has been documented on *Z. japonica*, and *Z. japonica* would be expected to provide this function, albeit on narrower leaves and higher in the intertidal than *Z. marina*[49].

Leaves of seagrasses slow down water movement and reduce currents and waves. These processes have been studied and quantified in several seagrass species but much remains unknown[16, 17, 18, 20, 28, 53]. In the PNW, one study measured water movement 32% greater in plots where *Z. japonica* had been removed[51]. Thus *Z. japonica* reduced currents and would be expected to reduce currents and wave energy in the PNW[16]. The amount of reduction will vary with the density and height of *Z. japonica* as well as with the current and wave environment[28].

As currents are reduced, suspended material in the water settles and remains in eelgrass beds. This function is also dependent on density, height, and other morphometric characteristics of the seagrasses[28]. As particulate material is trapped the level of the sediment surface may increase. This function of trapping of sediments may be a beneficial function or a detrimental function depending on location and desired use of the flats. A study in Willapa Bay reported colonization by *Z. japonica* of areas where ghost shrimp *Neotrypaea californiensis* were controlled resulting in a higher sediment level in areas where *Z. japonica* developed[14]. In the PNW *Z. japonica* traps suspended material which in turn contributes to greater water clarity.

The increased structure of seagrass leaves retards water movement off intertidal flats. This holds water on seagrass covered tide flats longer than on flats without seagrasses[39]. Desiccation during low tide is a strong factor in determining the suitability of intertidal habitats for a wide variety of fauna and flora. Thus, the retention of water on intertidal flats can make habitat suitable for a wide range of organisms that otherwise could not live there. In the PNW this phenomenon of retarding water flow off of intertidal flats has been observed by oyster and Manila clam growers and reported for *Z. japonica*[41].

Seagrass roots and rhizomes also help to stabilize the sediment. When the wasting disease caused decline of *Z. marina* in most parts of the north Atlantic, sediments that had been stable for many years were eroded away[52]. *Z. japonica* would be expected to have a similar role. However, *Z. japonica* does not have as robust a rhizome system as *Z. marina* and inter-annual variations in density and presence of *Z. japonica* may make this function less likely for *Z. japonica* in the PNW.

Oxygen is produced by seagrasses as they photosynthesize. Some of this oxygen moves out of the plant and dissolves into the water column[4]. During the night seagrasses respire and absorb oxygen from the water. In productive eelgrass systems the net movement of oxygen will be into the water. At a time when low oxygen in the water is of concern, this function of eelgrasses, including *Z. japonica* may be considered a beneficial function. In Padilla Bay, the oxygen concentration can reach over 200% saturation during bright days in spring and summer[7, 10]. The water with 200% saturation has been in contact with both *Z. marina* and *Z. japonica* and the contribution of *Z. japonica* is expected to be comparable to its distribution and rate of productivity[6, 7]. Some of the oxygen produced in photosynthesis moves down the eelgrasses and into the sediments around the roots and rhizomes[33]. Thus sediments in the rhizosphere are oxygenated whereas sediments without eelgrasses may be less aerobic or even anaerobic. Again, *Z. japonica* may be expected to oxygenate sediments in proportion to its productivity.

Seagrasses are able to absorb nutrients from both the water and the sediments and move these nutrients from leaves to roots/rhizomes and vice versa[40]. The net effect of this movement of nutrients will vary with time, season and location. During the warmer months of higher growth, nutrient demand is higher and eelgrass communities may absorb enough nutrients to measurably reduce the concentration in the water. In Padilla Bay, nitrate concentrations decreased up to 10 fold in a single tidal cycle when water flowed over eelgrass beds of both *Z. marina* and *Z. japonica*[7, 8]. Similarly, in Yaquina Bay, Oregon, *Z. japonica* habitats were net sinks for nitrate, ammonium, and dissolved reactive phosphate[29]. Thus, in the PNW, *Z. japonica* is likely to lower the concentration of nutrients in the water during the growing season. Nutrient concentrations in the sediment are also altered as *Z. japonica* changes the sediment bacteria involved in nitrogen cycling[44].

Another ecosystem function of seagrasses deals with pH of the water. As eelgrasses photosynthesize they remove CO_2 from the water. In doing so, they increase the pH, decreasing the acidity of the water. What role this may have on the larger water column is not known. But for animals living within the *Z. japonica* canopy, this may be an important mechanism for keeping the water at a pH suitable for growth. In Padilla Bay water flowing off both species of eelgrasses had substantially higher pH when flowing off the eelgrass beds than the bulk water from the Salish Sea flowing onto the eelgrass beds (Bulthuis, unpublished data). Similar to the consideration for oxygen production, the role of *Z. japonica* in providing this function will be in proportion to its productivity.

Thus, *Z. japonica* provides a variety of ecosystem functions in the Pacific Northwest similar to that provided by native eelgrass, *Z. marina*, and other seagrasses.

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The interaction of *Zostera japonica* with shellfish culture in the PNW

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Few studies address the interaction between shellfish production and *Zostera japonica*. These studies have the limitation of using small-size experiment units, which may or may not reflect the true interactions at the production level. For the purpose of this review, the impacts of *Z. japonica* on shellfish are divided into four sections: recruitment, post-recruitment survival, growth and fitness, and harvesting and sales.

Recruitment: There are limited studies specifically addressing *Z. japonica* and shellfish recruitment. For Manila clams in Willapa Bay, Tsai et al. (2010) found mixed results. The lowest recruitment numbers were where eelgrass was removed by hand; intermediate numbers were in *Z. japonica* plots, and highest numbers were where *Z. japonica* was removed by harrowing. Ruesink et al (in review) found no difference in settlement of manila clams between plots where *Z. japonica* was physically removed or left in place. Numerous other studies not specific to *Z. japonica,* however, indicate that submerged aquatic vegetation (SAV) may increase recruitment of shellfish. *Mercenaria* clams have enhanced recruitment in *Z. marina* (Peterson 1986). Recruitment of bay scallops and cockles, was increased over bare sediment in *Z. marina* (Carroll et al. 2012), *Codium (*Carroll et al. 2010) and *Caulerpa* (Gribben et al 2009), respectively. These studies postulated that SAV structures, by dampening water currents, promote higher deposition of larvae (Carroll et al. 2012). The impact of SAV on shellfish recruitment may vary by season. Tu Do et al. (2011) reported that lower cockle recruitment on sediment colonized by *Z. notlii* compared to bare sand in early summer, but the opposite in November. Overall, while there is evidence to support the role of eelgrass in zooplankton recruitment (Bostrom and Bonsdorff 2000), there is insufficient support to suggest *Z. japonica* either directly enhances or deters the settlement process for commercial clams in Washington.

Post-recruitment survival: There are several studies on early post-settlement effects of *Z. japonica* on shellfish. Patten (2012) found reduced levels of juvenile manila clams (<15 mm) in sites where *Z. japonica* had been chemically controlled, but no difference in immediate adjacent paired sites with or without *Z. japonica* (Patten, unpublished 2013). Ruesink et al. (in review) in a bay-wide survey of Willapa Bay found more adult *M. arenaria* in eelgrass-vegetated sites, but no difference in Manila clams. They also report no difference in juvenile clam densities in the fall between sites where *Z. japonica* was removed by hand or left in place. After seven months, however, both *R. philippinarum* and *M. arenaria* had lower densities on the sites with *Z. japonica*. In an experiment that included both gravel additions and eelgrass control, they found that in the presence of gravel *Z. japonica* reduced juvenile clam abundance.

The decoupling of juvenile clam populations from adult populations is common (Dethier et al. 2012). Ruesink et al. (in review) reported that for manila clams in Willapa Bay, this decoupling can be several orders of magnitude and is likely the result of post-settlement predation. The role of *Z. japonica* in preventing or enhancing these early predation losses is less clear. Tu Do et al. (2011) theorized that seagrass acts as a refuge against predation of large cockle, especial by birds, but for juvenile cockle it had the opposite effect because of increased biomass of infauna predators. In a study on the differences in benthic invertebrates that occurred with *Z. japonica* removal using an herbicide in Willapa Bay, Booth and Rassmussen (2011) found twice the level of the *Phyllodocida* and *Spionida* polychaetes in the vegetated plot compared to the treated plot. These genera of polychaetes are known to predate young clams. Crabs, fishes, waterfowl, shorebirds, seastars, and drilling snails are also major predators of young clams (Gillespie et al. 2012). Although the densities of some of these predators may increase with *Z. japonica* colonization, there are no data on how these populations changes affect juvenile clam populations. Studies in non-west coast estuaries indicate that SAV offers shellfish, like clams and cockles, protection from some predators (Gribben et al. 2009, Irlandi 1997, Irlandi and Peterson 1991, Peterson 1982).

Growth and fitness: Z. japonica reduces the number of juvenile and adult manila clams (Patten et al. 2012), total clam production (Patten et al. 2012), clam growth rate (Patten et al. 2012) and clam condition (Patten et al. 2012, Tsai et al. 2010). In a five-year study following the impact of *Z. notlii* colonization in France, Tu Do et al. (2012) found that manila clams, cockle and blue mussels all but disappeared from fully colonized sites, and cockle fitness declined. These suspension feeders were the only tropic group that did not show a high biomass after colonization. Similarly, shellfish growers in Willapa Bay cite incidences where oyster beds infested with thick *Z. japonica* fail to fatten adequately enough to be commercially harvestable. It is postulated that, because eelgrass vegetation limits hydrodymanics, it alters feeding/ filtration efficiency (Allen and Willams 2003, Tsai et al. 2010, Patten et al. 2012). A review of seagrass landscape effects on bivalves (Bostrom et al. 2006), however, indicated an array of positive or negative responses depending on species of bivalve and seagrass, patch size, and site location. East coast *Mercenaria* clams (Irlandi and Peterson, 1990), for example, have greater growth in seagrass than sand. This beneficial effect is due to seagrasses' baffling effect, which traps and slows food for suspension feeding (Peterson et al. 1984).

One of the major impacts of *Z. japonica* on shellfish is likely due to its effects on the sediment. *Z. japonica* changes the benthos from a net nitrogen source to a net sink (Larned 2003), increases dissolved organic matter (Hahn 2003), total organic matter (Harrison 1987, Lee et al. 2001, Posey 1988), and accumulation of fine sediments (Posey 1988, Patten et al. 2012). Similar findings are reported for the other small bladed eelgrass, *Z. noltii* (Clavier et al. 2011, Tu Do et al. 2012). This accumulation of silts and organics create a thicker surface muck layer (Posey 1988). There is also short-term nighttime anoxia in the shallow waters retained by the eelgrass during low tides, due to plant respiration and organic matter mineralization (sulfate reduction) (Clavier et al. 2011). This may result in an increase in surface sediment and rhizosphere sulfide (Clavier et al. 2011, Rosenberg 1991) to a level detrimental to some benthic organisms, including bivalves (Clavier et al. 2011, Tu Do et al. 2012, Vinther et al. 2008; Booth and Heck 2009). These conditions, if short-term, may not be overtly toxic to Manila clams, but will reduce their glycogen levels (Kozuki et al. 2012) and cause them to dwell closer to the surface, where they are more susceptible to crab predation (Munari 2012). Reduced sediment condition can also result in increased manganese levels, potentially to a level that is neurotoxic to clams (Beirao & Nascimento 1989, Tsutsumi 2006). Finally, just the increase in sediment silt composition alone may dramatically suppress manila clam growth (Melia et al 2004), or bury the supplemental surface gravel (Patten et al. 2012) used for clam production in many PNW estuaries.

Disease and parasites are biotic stressors that diminish shellfish populations (Gillespie et al. 2012). Short-term anoxia could enhance these stressors. *Z. noltii* colonization in France, for example, increased the trematodes assemblages infecting cockle four-fold and may be partially accountable for the decline in cockle fitness (Tu do et al. 2012). There are no studies, however, in the PNW on the effects of Z. *japonica* on shellfish diseases and/or parasites.

Biofouling from drift algae can cause anoxia and crop loss in Manila clams (Adams et al. 2011). In Willapa Bay, growers report increased problems at sites that are colonized by *Z. japonica*. Although there is no research on Z. Japonica, artificial *Ruppia maritima* and *Z. marina have* been noted to increase drift algae coverage of sediment (Bostrom and Bonsdorff, 2000).

Harvest and sales: For hand harvest of Manila clams in Willapa Bay, growers report a 5% crop loss due to difficulty of finding and removing clams in *Z. japonica* infested beds, and an extra $0.05/lb in cleaning cost (Patten, 2012). Growers also report they are unable to use a machine harvester in densely infested *Z. japonica* beds. Net losses to Manila clam farmers in Willapa Bay, based on extrapolation from small plot data, ranged from $2,700 to $17,000/ac/ harvest cycle, depending on site productivity (Patten 2012). Finally, although not studied under Washington conditions, there is a possibility of lost sales due to toxic dinoflagellates buildup. In France, Genovesi et al. (2013) found that long-lived resting cysts of HAB dinoflagellates favor sites with fine sediments and higher organic matter over sandy substrates. These are the conditions induced by *Z. japonica* colonization (Posey 1988).

Summary: In the studies cited herein, there is ample evidence that *Z. japonica* has credible economic impact to the shellfish industry in Washington estuaries. The unique biotic and abiotic components of each estuary, and the importance of the scale of eelgrass colonization (To Du et al. 2012), however, make inferences on the impacts of *Z. japonica* on shellfish both site and temporal specific. To help fill existing gaps in data, including impacts on oyster production, predation, diseases and parasites, short-term anoxic stress, toxicity of anaerobic sediments, and bio-fouling, there is a need for large-scale, long-term replicated paired studies.

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Interactions between *Zostera japonica* and estuarine fauna in the Pacific Northwest

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As a primary producer and through its biogenic structure, non-native dwarf eelgrass (*Zostera japonica*) is expected to support higher abundance and diversity relative to unstructured tideflats. Predictions in comparison to native *Z. marina* are not straightforward, particularly as the species frequently occupy different intertidal zones that could confound results. Methods to collect epifauna include submerged seagrass collections or epibenthic pumps. Epifauna should respond positively to structural complexity, but their predators could either be attracted to this resource or kept out by structure. Infauna are usually collected in cores. Protection and organic material provided by *Z. japonica* could increase infaunal abundance, but boundary layer effects could reduce gas and resource exchange and therefore reduce infaunal production. In any case, altered composition may be associated with *Z. japonica* relative to unstructured tideflats. In Hong Kong, where *Z. japonica* is native, infauna and especially epifauna have higher richness and abundance in *Z. japonica* than unstructured tideflats (Lee et al. 2001).

***Z. japonica* and infauna – comparison to unstructured tideflats:**

Location	Method	Replication	Result with Zj	Reference
Roberts Bank, BC	Natural expansion of Zj	1977-1984	Burrowing shrimp decline	Harrison 1987
Roberts Bank, BC	Addition of Zj, Removal of Zj in 0.25 m^2	N=5, N=20, N=20 x 2	Fewer burrowing shrimp and tubeworms	Harrison 1987
Coos Bay	Cores inside and outside Zj patches	N=5 at 3 sites and 9 times	Infauna SR higher due to lower-elevation taxa; Higher abundances of 10/11 dominant taxa	Posey 1988
Coos Bay	Addition of Zj to $1m^2$, compared to disturbance control and control	N=7, initiated at different times	3 species more abundant, 1 species less so, 3 unchanged	Posey 1988
Boundary Bay	Cores in existing habitat types across elevations	3 cores every 100 m along 2 transects	Molluscs eaten by birds asserted to be more abundant in eelgrass, but no obvious analysis	Baldwin and Lovvorn 1994 MB

Netarts Bay	Reciprocal shrimp and Zj transplants, bottomless buckets	N=5 in each of 4 treatments	Fewer shrimp burrows, at least in mid-summer	Berkenbusch et al. 2007
Willapa Bay	Removal of Zj in 100 m^2	N=5	Lower condition of Manila clams	Tsai et al. 2010
Willapa Bay	Removal of Zj in 20x20 m	One plot, 12 cores in and outside	No difference: Abundance, richness, diversity higher in Zj both before and after treatment	Booth et al. 2011

***Z. japonica* and infauna – comparison across multiple habitats:**

Locatio n	Method	Replicatio n	Result with Zj	Referenc e
Grays Harbor	Cores in existing habitat types	N=15-20, 0.01 m^2 cores	Richness, abundance, biomass higher relative to unstructured, but not Zm; Significant Bray-Curtis dissimilarity to all other habitats	Ferraro and Cole 2011
Tillamo ok Bay	Cores in existing habitat types	N=15-20, 0.01 m^2 cores	Higher richness, abundance, and biomass than most other unstructured habitats and Zm; Significant Bray-Curtis dissimilarity to all other habitats	Ferraro and Cole 2012

***Z. japonica* and epifauna – comparison across habitats:**

Location	Method	Replication	Result	Reference
Padilla Bay	0.25m^2 plots in existing habitat types across elevations	See below	YOY Dungeness crab – see below	Dinnell et al. 1986
Padilla Bay	Epibenthic pump and “pitfall” traps of incoming tide’	N=3 at 0.5’(Zm), 1.2’ (Zj), 4.5’ (mud)	No statistical analysis: Epibenthic abundance similar but richness higher in vegetation; Benthic boundary layer abundance and richness higher in Zm than Zj and mud	Simenstad et al. 1988
Boundary Bay	Sweep nets in existing habitat types across elevations	0.3 m^3 at intervals	Several amphipods increase in abundance – vegetation (Zj, Zm) is usually better predictor than tidal	Baldwin and Lovvorn 1994 MB

			elevation	
Padilla Bay	Collect shoots from existing habitat types across elevations	3 samples in each of 3 zones at 7 times	Caprellid amphipods, isopods, snails increase with shoot density from Zm to Zj	Thom et al. 1995

***Z. japonica* and fish:** Chinook salmon hatchery smolts in a 4000 m^2 field enclosure reduced swimming speed and preferred *Z. marina* patches relative to other habitats including *Z. japonica* and unstructured (Semmens 2008).

***Z. japonica* and birds:** *Z. japonica* constituted large fractions of the dry mass of esophageal contents for birds in Boundary Bay: American wigeon (85%, n=45), northern pintail (48%, n=54), mallard (72%, n=20), green-winged teal (2%, n=14), and brant (57%, n=62; Baldwin and Lovvorn 1994MB).

Effects ON *Z. japonica* from estuarine fauna: Effects may occur through ecosystem engineering and competition: The removal of burrowing shrimp (*Neotrypaea californiensis*) in Willapa Bay improved seedling survival (Dumbauld and Wyllie-Echeverria 2003), although in Netarts Bay the same shrimp species had no negative effect on transplanted adult *Zostera japonica* from April to August (Berkenbusch et al. 2007). Facilitative effects among non-native species are also possible: Intertidal plots with introduced hornsnails (*Batillaria attramentaria*) had more *Z. japonica* than those without (Wonham et al. 2005). Consumption of *Z. japonica* could result in top-down control, however, this interaction does not seem coincident with most rapid growth of *Z. japonica*: Birds in Boundary Bay were estimated to consume 51% of aboveground and 43% of belowground biomass of *Z. japonica* while overwintering in 1991-1992 (Baldwin and Lovvorn 1994MEPS), and likely switched seasonally to forage in farmland due to depletion of resources on tideflats (Lovvorn and Baldwin 1996).

Young-of-year Dungeness crab: Quadrats of 0.25 m^2 were excavated in different habitat types across elevations in Padilla Bay and sieved. Two samples during peak recruitment give an indication of habitat associations (Dinnell et al. 1986):

Habitat	No. quadrats, 25 Aug 1986	Mean m^{-2} YOY Dungeness crab	No. quadrats, 10 Sept 1986	Mean m^{-2} YOY Dungeness crab
Z. japonica	4	1	3	55
Z. marina	33	8	3	0
Z. marina + algae	4	1	7	11
Ulva	14	10	6	37
No plant cover	2	0	3	4

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Interactions between *Z. japonica* and *Z. marina*

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The few studies of the interactions between *Zostera marina* and *Zostera japonica* suggest a complex, context-dependent relationship. The relative intertidal zonation of these two species varies from site to site, and remains a subject of study. In studies which distinguished biotic interactions from abiotic influences, competition has been evident, and the effects of *Z. marina* on *Z. japonica* appear more consistent than the reciprocal.

An examination of Puget Sound-wide monitoring data suggests that co-occurrence of *Z. marina* and *Z. japonica* is most likely at gently sloped beaches with smooth depth profiles (Hannam, 2013). The same study found that *Z. marina* occurs at higher elevations in the Puget Sound at sites where *Z. japonica* was observed.

Harrison (1982) grew *Z. marina* and *Z. japonica* separately in mesocosm under different simulated tidal regimes, and seasonal light regimes. Both species had higher leaf elongation rates in simulated subtidal conditions, than when exposed during a low tide. When continuously submerged, both species had similar leaf elongation rates in simulated spring light and temperature conditions, but *Z. marina* outgrew *Z. japonica* in warmer, brighter conditions with longer day length. When exposed during low tides, leaf growth was similar between the two species.

In a study in Roberts Bank, British Columbia Nomme and Harrison (1991a) examined morphological traits of *Z. marina* and *Z. japonica* at depths where each species occurred in monocultures and at a depth where both species co-occurred. *Z. japonica* shoot density remained low throughout the growing season where it was observed growing with *Z. marina*, but increased exponentially, before decreasing late in the season, where growing monospecifically. *Z. marina* shoot density did not differ between monospecific and mixed stands. Multivariate analysis of morphological traits of each species detected differences between elevation zones on some, but not all, dates of the study. This study did not distinguish between effects of tidal elevation per se and the biotic effect of co-occurring with a congener.

In a different study at Roberts Bank, Nomme and Harrison (1991b) found progressively reduced *Z. japonica* shoot density where *Z. japonica* was transplanted to deeper depths in monoculture. Both species grew longer leaves at deeper sites, but *Z. marina* shoot densities were unaffected by transplant elevation.

Manipulative studies of *Z. marina* and *Z. japonica* have consistently found *Z. japonica* to be competitively suppressed by *Z. marina* presence, although the mechanisms remain unclear. Hahn (2003b) transplanted sods from existing mixed-species and monospecific stands to tidal elevations in the *Z. japonica* zone, the *Z. marina* zone, and their overlapping zone. *Z. japonica* shoot densities were reduced by approximately 50% at all elevations where growing with *Z. marina*. Bando

(2006) conducted a replacement transplant experiment, using individual shoots of *Z. marina* and *Z. japonica* at a set density. *Z. japonica* biomass per individual was reduced by 96% in mixed transplants versus monoculture. Hannam (2013) conducted an additive experiment, transplanting arrays or shoots of each species in monocultures and with its congener onto intertidal mounds and pools. *Z. marina* density and biomass were profoundly decreased on mounds, but unaffected by *Z. japonica*. *Z. japonica* was, suppressed by *Z. marina* presence, more so in pools than on mounds.

Manipulative studies have sometimes detected competitive effects of *Z. japonica* on *Z. marina*. Merrill (1995) found an increase in *Z. marina* shoot elongation in response to clipping *Z. japonica* shoots. Hahn (2003b) *Z. marina* found that shoot densities were lower in mixed plots than in monocultures, but only in the deeper elevations. Merrill (1995) found an increase in *Z. marina* shoot elongation in response to clipping *Z. japonica* shoots. Bando (2006) reported reduced *Z. marina* above-ground biomass per individual in response to *Z. japonica* transplantation, but the graphically presented data in the paper contradict this conclusion, showing greater *Z. marina* biomass per individual in two-species plots. Hannam (2013) found decreased branching and rhizome elongation in *Z. marina* transplanted into *Z. japonica* in tide pools, but not on intertidal mounds.

Z. japonica has been quicker to recolonize experimentally disturbed sites whenever studies have addressed this (Hahn, 2003a; Bando, 2006). Such a finding is congruent with observations that *Z. japonica* devotes more to sexual reproduction than does *Z. marina*, and that *Z. japonica* is quick to recolonize disturbed areas (Park et al., 2011). *Z. marina* recolonization often proceeds at a slower pace, and may be more reliant on rhizome expansion than seed rain (Boese et al., 2009).

Where *Z. marina* and *Z. japonica* co-occur, they compete. *Z. marina* appears to be the dominant competitor, and *Z. japonica's* competitive effects on *Z. marina* are not evident at sites where abiotic conditions stress *Z. marina*. *Z. japonica's* dispersal and colonization abilities should allow it to coexist with *Z. marina* and thrive where disturbance is common. Some studies suggest that *Z. japonica* could facilitate *Z. marina* survival at higher tidal elevations, but this hypothesis remains largely untested.

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Appendix

The Science and Management of *Zostera japonica* in Washington: A meeting for state agencies:

Meeting description and agenda

MEETING DESCRIPTION

The Science and Management of *Zostera japonica* in Washington: A meeting for state agencies

June 18 – 19, 2013
Tuesday 8:30 am – 4:00 pm
Wednesday 8:30 am – 3:00 pm

Lacey Community Center, 6729 Pacific Ave. SE

Co-sponsors: Padilla Bay National Estuarine Research Reserve, Washington Department of Ecology, Washington Department of Natural Resources, Washington Department of Fish and Wildlife, Washington Invasive Species Council.

Purpose: To improve state agency understanding of *Zostera japonica* science and management in Washington State.

- *Objective 1.* Review the science on the ecology of *Z. japonica* in Washington State, including the results of the September 2010 workshop (attached) and studies and research completed since 2010.
- *Objective 2.* Review the existing *Z. japonica* science as it relates to specific policy and management questions.
- *Objective 3.* Provide an opportunity for state resource managers and policy staff to discuss the challenges and options for managing *Z. japonica*, and identify any action items that would increase coordination among state agencies.
- *Objective 4.* Document the outcome of the meeting in three ways:
    1. A fact sheet summarizing the current state of the science (to be created by the Department of Natural Resources)
    2. A compilation of presenter abstracts and science panel report (to be created by Padilla Bay Reserve)
    3. Summary of policy discussion and outcomes (to be created by the Department of Ecology)

Facilitator:

Jerry Thielen (Thielen Consulting) will assist in fostering a productive and informative dialogue regarding this important natural resource issue. Jerry has a BS in Environmental Studies (WSU) and retired from Ecology in 2009 as the Regulatory Affairs Manager. In that position, Jerry oversaw all regulation development and was involved in legislative policy review and implementation, including training and coordinating public hearing officers. Jerry has a strong background facilitating public meetings that engage broad interests such as scientists, governments, environmentalists, industry and other stakeholders.

Jane Dewell of the Governor's Office of Regulatory Assistance will assist Jerry. Jane has a BA in Zoology (UW '81) and MS in Environmental Studies (UMT '87). She has planned and coordinated many public forums and facilitated a broad range of public and technical meetings on environmental issues. She currently manages the multi-agency review of the proposed Cherry Point coal shipping terminal.

The Science and Management of *Zostera japonica* in Washington

AGENDA

Day 1: Tuesday, June 18, 8:30 – 4:00

8:30 – 9:00 Coffee and sign in; meet the speakers

9:00 – 9:30 Welcome

Introductions, meeting objectives and ground rules
- Jerry Thielen, Facilitator

Host Welcome and Meeting Purpose
- Gordon White, WA Dept. of Ecology

Regulatory Overview Handout, Sharing Meeting Results and Future Opportunities
- Cedar Bouta, WA Dept. of Ecology

9:30 – 12:15 Science Presentations (includes a 45-minute lunch break)
Introduction of panel and speakers by Doug Bulthuis,
Padilla Bay National Estuarine Research Reserve

9:30 – 9:45 Summary of 2010 workshop
Jeff Gaeckle, Ph.D., WA Dept. of Natural Resources
9:45 – 10:30 *Z. japonica* distribution and spread in the Pacific Northwest
Jim Kaldy, Ph.D., US EPA
10:30 – 11:00 Role and growth of *Z. japonica* in its native range
Fred Short, Ph.D., WA Dept. of Natural Resources
11:00 – 11:45 Ecosystem functions of *Z. japonica* in the Pacific Northwest
Doug Bulthuis, Ph.D., Padilla Bay
11:45 – 12:15 Questions from the audience?

12:15 – 1:00 LUNCH (provided)

1:00 – 3:45 Science Presentations (continued)
Introduction of speakers by Fred Short, Department of Natural Resources

1:00 – 1:45 *Z. japonica* and shellfish culture
Kim Patten, Ph.D., WSU Cooperative Extension
1:45 – 2:30 Interactions between *Z. japonica* and estuarine fauna in the Pacific Northwest
Jennifer Ruesink, Ph.D., University of Washington
2:30 – 3:15 Interactions between *Z. japonica* and *Z. marina*
Michael Hannam, Ph.D., University of Washington
3:15 – 3:45 Questions from the audience?

3:45 – 4:00 Summary of day one results and accomplishments; overview of day two (Jerry Thielen)

The Science and Management of *Zostera japonica* in Washington

AGENDA

Day 2: Wednesday, June 19, 8:30 – 3:00

8:30 – 9:00 Coffee and sign in; meet the panelists

9:00 – 9:30 Welcome

Introductions, meeting objectives and ground rules
- Jerry Thielen, Facilitator

Host Welcome and Meeting Purpose
- Gordon White, WA Dept. of Ecology

Regulatory Overview Handout, Sharing Meeting Results and Future Opportunities
- Cedar Bouta, WA Dept. of Ecology

9:30 – 9:50 Introduction of state agency representatives and perspectives (Jerry Thielen)

9:50 – 11:30 Science Panel

9:50 – 10:00 Introduction of panelists by Fred Short, Department of Natural Resources
- Mary O'Connor, Ph.D., Dept. of Zoology, University of British Columbia
- Brett Daumbauld, Ph.D., Oregon State University & US Dept. of Agriculture
- Debra Shafer, Ph.D., Army Corps of Engineers, Mississippi
- Steve Rumrill, Ph.D., Oregon Dept. of Fish Wildlife, Shellfish Program Leader
- Renee Takesue, Ph.D., USGS Pacific Coastal & Marine Science Center, California

10:00 – 10:30 Overall summary of science on Z. japonica
Report on the panel's evaluation of the strength of science

10:30 – 11:30 Q and A

11:30 – 12:15 LUNCH (provided)

12:15 – 2:45 Policy Discussion for State Agencies:
The challenges of managing *Z. japonica* and possible next steps

How do we move forward collaboratively to gain clarity, consistency, and improve coordination across state agencies regarding the management of *Z. japonica*?
- What additional information needs to be gathered?
- What are our next steps? When and how will we check back to evaluate the effectiveness of this approach?

2:45 – 3:00 Closing: Review Action Items